

mutation rate. This indirect selective force is considerably weakened by genetic recombination, a process that breaks apart linked genes — and a factor not included by Kamp *et al.* in their model. But recombination is substantial in many viruses, and its effect should probably be considered explicitly in modelling the evolution of viral mutation rates.

Despite these shortcomings, the paper of Kamp *et al.*² is clearly an important conceptual development in the study of mutation-rate evolution in viruses. Moreover, developing a fuller understanding of the evolutionary causes and consequences of viral mutation rates is worthwhile from both basic and applied perspectives. Drugs that increase genomic mutation rates can kill off viral populations by causing them to exceed their error threshold^{9,10}. A quantitative theory that can predict how close to the error threshold a given viral population is — without the need to estimate its

mutation rate directly — might have real therapeutic value. ■

Sebastian Bonhoeffer is in the Department of Environmental Science, ETH Zürich, CH-8092 Zürich, Switzerland.

e-mail: bonhoeffer@eco.unm.w.ethz.ch

Paul Sniegowski is in the Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.

e-mail: paulsnie@sas.upenn.edu

1. Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. *Genetics* **148**, 1667–1686 (1998).
2. Kamp, C., Wilke, C. O., Adami, C. & Bornholdt, S. *Complexity* (in the press); Preprint cond-mat/0209613 (2002). <http://arXiv.org>
3. Eigen, M. *Naturwissenschaften* **58**, 465–523 (1971).
4. Kimura, M. *Genet. Res.* **9**, 23–34 (1967).
5. Leigh, E. G. *Genetics* **73**, 1–18 (1973).
6. Ishii, K., Matsuda, H., Iwasa, Y. & Sasaki, A. *Genetics* **121**, 163–174 (1989).
7. Nowak, M. A. *Nature* **347**, 522 (1990).
8. Sniegowski, P. D., Gerrish, P. J., Johnson, T. & Shaver, A. *BioEssays* **22**, 1057–1066 (2000).
9. Loeb, L. A. *et al. Proc. Natl Acad. Sci. USA* **96**, 1492–1497 (1999).
10. Crotty, S., Cameron, C. E. & Andino, R. *Proc. Natl Acad. Sci. USA* **98**, 6895–6900 (2001).

Plant biology

Fixation with regulation

J. Allan Downie and Martin Parniske

A gene has been isolated that controls the number of symbiotic nitrogen-fixing nodules in legumes. Its similarity to a well-characterized regulatory gene in *Arabidopsis* provides clues about its action.

Leguminous plants produce root nodules, within which symbiotic bacteria capture atmospheric N₂ and convert it into nitrogen that can be used by the plant. But this process is energetically expensive and so legumes strictly control the numbers of nodules they form. Papers by Krusell *et al.*¹ and Nishimura *et al.*² (pages 422 and 426 of this issue), and by Searle *et al.*³ in *Science*, describe the characterization of a regulatory gene that normally limits nodule numbers, and that when mutated increases nodulation. Control of nodule development is of interest in its own right, but this work may also have agricultural applications.

Soybean and pea mutants with enhanced nodulation have been known for about 20 years⁴, but their complex genomes have hampered attempts to clone the genes responsible. Similar mutants^{5,6} were recently identified in *Lotus japonicus*, a legume with a relatively small genome. These mutants have hypernodulation and aberrant roots, hence their designation as *har* mutants. The numbers of both nodules and lateral roots are increased in these *L. japonicus* and soybean mutants, indicating that normal legumes possess a common regulatory mechanism that limits the numbers of root and nodule growing points, or meristems.

Grafting experiments showed that *HARI* control of nodule and lateral-root number

in *L. japonicus* depends on the shoots rather than the roots (Fig. 1, overleaf), a characteristic that had been previously observed with soybean hypernodulation mutants⁴. So plants with *har1*-mutant shoots grafted onto wild-type roots had increased root nodulation. In contrast, the reciprocal grafted plants (mutant roots with wild-type shoots) had normal roots and nodules. After positioning *HARI* on a physical map of the *L. japonicus* genome, two groups^{1,2} cloned the gene. They went on to identify mutations in the equivalent genes in pea¹ and soybean² hypernodulation plants, which showed a similar shoot control of nodulation³. Independently, following about 15 years of work³, the equivalent gene controlling nodulation in soybean was isolated and was called *NARK* (nodule autoregulation receptor kinase). It is clear that *HARI* and *NARK* are the same genes from different species.

HARI and *NARK* encode a type of receptor protein that is abundant in plants⁷ and has three components: an extracellular domain of leucine-rich repeats, a membrane-spanning domain, and an intracellular protein kinase domain (Fig. 2). This structure is compatible with the receptor's function being perception of a ligand outside the cell, followed by internal signal transduction through protein phosphorylation by the kinase domain. Mutant genes sequenced from the three species had



100 YEARS AGO

We have received from Messrs. J. W. Gray and Son a pamphlet on scientific protection against lightning, written by Mr. A. Hands. The writer gives a careful explanation of the principles which must be observed in erecting lightning conductors; as the pamphlet is written in non-technical language, it is to be hoped it may be the means of disseminating information amongst the public, since there are few subjects on which more ignorance and superstition exist. The importance of careful protection may be gathered from the fact that Mr. Hands estimates the damage caused annually by lightning in this country alone at from 50,000*l.* to 100,000*l.*

ALSO

The Liverpool correspondent of the *Central News* states that the Nobel prize of 3,000*l.* for researches in connection with malaria will be a personal one to Major Ross, principal of the Liverpool School of Tropical Medicine. According to the Stockholm correspondent of the *Daily Chronicle*, the prize for medicine will be awarded to Prof. Finsen, the Danish discoverer of the treatment by red light for lupus, and the prize for physics to Prof. S. A. Arrhenius. From *Nature* 27 November 1902.

50 YEARS AGO

On November 4 at 16h. 58m. 20s. G.M.T., an earthquake occurred with epicentre... near the east coast of Kamchatka. It was recorded strongly at seismological observatories throughout the world and had a magnitude of 8¹/₃ on the Gutenberg–Richter logarithmic scale... Faulting probably took place in the sea bed near the epicentre since a great tsunami or seismic sea wave resulted, and spread throughout the Pacific Ocean. It arrived at the coast of northern Japan about 20h. G.M.T. on November 4. The Hawaiian warning system was used and the coastlines of several islands, including the Oahu coast, were evacuated in anticipation of the wave. When the wave arrived at Hawaii itself, it is reported to have been several feet high... Waves from one to three feet high arrived at the Whangarei beaches in the north of New Zealand about 7 p.m. local time on November 5. When these waves arrived at Wellington, they were 6–8 in. high... Immediately following the main shock, there were more than a hundred aftershocks. Further news is awaited.

From *Nature* 29 November 1952.

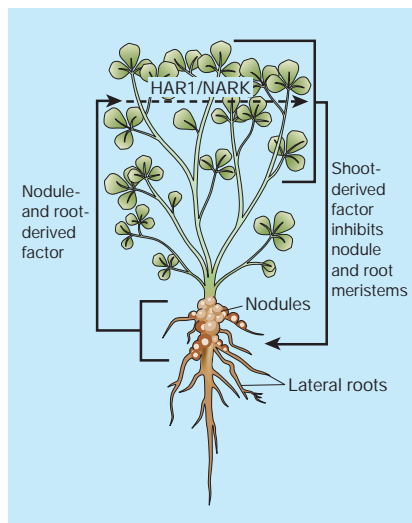


Figure 1 Control of nodule number in legumes. One proposal¹ is that a factor produced by nodule and root meristems is detected in shoots, resulting in the production of a second factor that suppresses the development of new nodules. In line with this model, mutation of the *HAR1/NARK* gene increases the numbers of nodules and lateral-root meristems^{1–3}. So *HAR1/NARK* protein is required for the production of the shoot-derived inhibitor, possibly through its involvement in perception of a nodule- and root-derived signal.

alterations in the kinase domain, suggesting that phosphorylation by *HAR1/NARK* protein is essential for signalling. The signal perceived by *HAR1/NARK* is unknown, as is the kind of inhibitor that suppresses further nodulation.

Comparison with the model plant *Arabidopsis* provides insight into how *HAR1/NARK* might function, even though *Arabidopsis* does not form nodules. The *Arabidopsis* genome is predicted to contain 216 leucine-rich-repeat, receptor-like kinases⁷. They are believed to perceive extracellular signals such as bacterial flagellin, the wounding signal systemin, and the plant hormones brassinolide and phytosulphokine. A real surprise from the new work^{1–3} is that the legume *HAR1/NARK* is more similar in sequence to *Arabidopsis* *CLAVATA1* than to any other *Arabidopsis* protein, implying that, like *CLAVATA1*, it functions as a receptor kinase. Together with *CLAVATA2*, *CLAVATA1* forms a complex that detects the signal peptide *CLAVATA3* (Fig. 2). The *CLAVATA* genes are involved in regulating cell fate in the shoot apical meristems⁸, *clavata1* mutants having an enlarged meristematic zone that leads to fasciation (contiguous parts growing into one).

CLAVATA1 and *HAR1/NARK* appear to have similar functions, because mutation of the corresponding genes results in increased meristematic activity (Fig. 2), either in shoot apical meristems (*clavata1*) or in root and

nodule meristems (*har1/nark*). However, they must have different roles, because the roots of *clavata1* mutants are unaffected, as are the apical meristems of *nark* mutants³. Moreover, their patterns of expression are different. *CLAVATA1* is exclusively expressed in apical meristems⁸, whereas *HAR/NARK* seem to be expressed in most tissues except apical meristems^{2,3}. It will be interesting to see how far the different functions of *HAR1/NARK* and *CLAVATA1* can be attributed to their expression patterns.

Grafting and other experiments with soybean hypernodulation mutants led to a model of how legumes might regulate nodule number⁴. This postulates that a signal moves from the root to the shoot, and that increased root nodulation is detected in shoots as the root-derived signal increases. As a result, the shoots produce an inhibitor, which is translocated to the root, where it represses further nodule development. Based on the grafting experiments and the nature of *HAR1/NARK*, it seems that this protein could be involved in the perception of a root-made signal in the shoot (Fig. 1). An unexpected result is that all three groups^{1–3} detected *HAR1/NARK* messenger RNA in roots, implying that the protein is being made there. But although the gene is transcribed in roots, the grafting experiments show that the root-expressed gene alone cannot function in nodule repression. So some additional factor in the aerial part of the plant may be required for *HAR1/NARK* to act.

There is a second shoot-controlled hypernodulation gene in pea that, when mutated, can lead to shoot fasciation⁹. Given the similar fasciation seen with the *clavata* mutants, there may be some functional overlap of the *CLAVATA* and *HAR1/NARK* pathways. It seems likely that the product of this second gene in pea could be part of the same signalling pathway as *HAR1/NARK*. Identification of the gene will be required to see if its product can interact directly with *HAR1/NARK* (Fig. 2) and what relationship there is between the *HAR1/NARK* and *CLAVATA* pathways. Genes with high sequence similarity to *CLAVATA1* have been identified in soybean¹⁰, and so *HAR1/NARK* may have arisen as a duplication of an ancestral *CLAVATA1*-like gene, with a subsequent divergence of functions.

Finally, the results of Krusell *et al.*¹, Nishimura *et al.*² and Searle *et al.*³ have practical implications. One of the problems with most of the existing hypernodulation mutants is that the abnormal root and nodule development has severe effects on plant growth. However, Searle *et al.*³ describe one mutation in *NARK* that has less detrimental effects. A different *L. japonicus* gene encoding a transcriptional regulator that represses nodulation has also just been described¹¹, but its relationship to the *HAR1/NARK* receptor pathway is not known. Now that

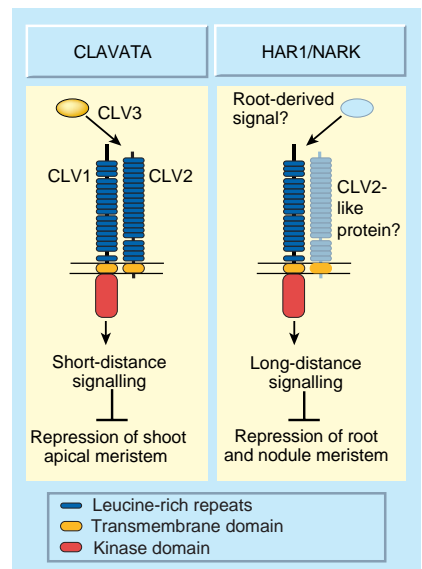


Figure 2 How *HAR1/NARK* might function in signalling and root nodulation. This scheme is based on the model⁸ for signalling by *CLAVATA* proteins in *Arabidopsis*, and on the characteristics of *HAR1/NARK* described by Krusell *et al.*¹, Nishimura *et al.*² and Searle *et al.*³. *HAR1* is most similar to *CLAVATA* (*CLV*) 1, a receptor kinase containing leucine-rich repeats which interacts with *CLV2*, forming a complex that recognizes the signalling peptide *CLV3*. *CLV2* contains leucine-rich repeat and transmembrane domains, but lacks a kinase domain. *HAR1/NARK* might interact with a *CLV2*-like protein, yet to be identified, to recognize a signal produced by root and nodule meristems.

key genes regulating nodulation have been isolated, it may be possible to identify plants carrying subtle mutations — these might allow increased nodulation, with consequent increases in nitrogen fixation, without affecting plant growth too badly. Such plants might show improved growth, and also leave residual nitrogen in the soil to increase the growth of subsequent crops. ■

J. Allan Downie is in the Department of Molecular Microbiology, and Martin Parniske is in the Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

e-mails: allan.downie@bbsrc.ac.uk
martin.parniske@sainsbury-laboratory.ac.uk

1. Krusell, L. *et al.* *Nature* **420**, 422–426 (2002).
2. Nishimura, R. *et al.* *Nature* **420**, 426–429 (2002).
3. Searle, I. R. *et al.* *Science* published online 31 October 2002 (doi: 10.1126/science.1077937).
4. Caetano-Anolles, G. & Gresshoff, P. M. *Annu. Rev. Microbiol.* **45**, 345–382 (1991).
5. Wopereis, J. *et al.* *Plant J.* **23**, 97–114 (2000).
6. Kawaguchi, M. *et al.* *Mol. Plant–Microbe Interact.* **15**, 17–26 (2002).
7. Shiu, S.-H. & Bleecker, A. B. *Proc. Natl Acad. Sci. USA* **111**, 10763–10768 (2001).
8. Clark, S. E. *Nature Rev. Mol. Cell Biol.* **2**, 276–284 (2001).
9. Sagan, M. & Duc, G. *Symbiosis* **20**, 229–245 (1996).
10. Yamamoto, E., Karakaya, H. C. & Knap, H. T. *Biochim. Biophys. Acta* **1491**, 333–340 (2000).
11. Nishimura, R., Ohmori, M., Fujita, H. & Kawaguchi, M. *Proc. Natl Acad. Sci. USA* **99**, 15206–15210 (2002).